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DATE: July 3, 2001

SUBJECT: BRAND NAME: Tamaron 600 SL
ACTIVE INGREDIENT: Methamidophos
COMPANY NAME: Bayer Corporation
TRACKING I.D. NUMBER: 184857
RECORD NUMBER (RN): 176927
DATA PACKAGE NUMBER (DPN): 315-164
EPA REGISTRATION NUMBER: 3125---
TITLE: Absorption, Excretion, Balance and Pharmacokinetics of ^{14}C Radioactivity after Single Dose Dermal Application of One Dose Level of ^{14}C Labeled Methamidophos from a Tamaron 600 SL Formulation Administered to Healthy Volunteers

A dermal absorption study of methamidophos in six healthy male volunteers was conducted by Pharma Bio-Research Clinics, B.V (PBR) of The Netherlands (Clinical phase) and XenoBiotic Laboratories, Inc (XBL) of the United States (Analytical phase). This study was initiated on November 20, 1998 and was completed on July 11, 2000. All aspects of this study, which were performed at Pharma Bio-Research Clinics, were conducted in accordance with Good Clinical Practice Regulations. The conditions were in compliance with the Declaration of Helsinki (with subsequent revisions) and other guidelines on the use of human subjects. All aspects of this study, which were performed at XenoBiotic Laboratories, Inc., were conducted in accordance with the U.S. EPA FIFRA Good Laboratory Practice Standards (40 CFR Part 160). The quality assurance officer inspected various aspects of the study. A summary of this dermal absorption study and the evaluation of the results are presented below.

A. Preparation of Test Subjects

Human volunteers were recruited into the study through the advertisement in English and Dutch. The eligibility screening of the volunteers was conducted within three weeks before initiation of the study. Six healthy male volunteers were selected from a pool of applicants. Consent forms were voluntarily signed by each participant and all were thoroughly instructed as to the nature and objectives of the study. An attending physician and other staff provided the care to volunteers throughout the study. All subjects were housed within the Pharma Bio-Research

Clinical Research Center for the duration of the study. The mean body weight of the volunteers was 80.9 ± 6.6 kg.

Preliminary baseline urinalysis, hematology and blood chemistry were evaluated. The application site was outlined with ink on the day of dosing and surrounded by an adhesive template (Duoderm®, Squibb B.V., The Netherlands) from which a 4 x 6-cm section had been removed. An indwelling intravenous (IV) catheter was placed in both arms for simultaneous collection of blood samples for the first 8 hours of the study.

B. Preparation and Administration of the Dose

An appropriate amount of [$^{14}CH_3S$]-methamidophos was mixed with Tamaron 600 SL blank formulation. The formulation was diluted with water to obtain a concentration of 72 µg/100 µL. The six volunteers were administered a single 100 µL dose of [$^{14}CH_3S$]-methamidophos in the Tamaron 600 SL formulation. The mean dose of 71 µg of radiolabeled methamidophos was applied topically to an area of 24 cm^2 equivalent approximately to 3 µg a.i./cm^2. The applied area was the non-dominant volar (palmar) aspect of the antebrachium of each subject. This dose was selected because it represented the approximate exposure experienced by agricultural workers. After administration of the dose, the site was covered with a porous aluminum dome secured with an adhesive bandage. This allowed air to circulate but avoided loss of test article due to physical contact. Normal daily activity was allowed inside the research center and standard meals were provided at regular hours. Bathing and showering were not allowed until after the tape stripping on day 3 in order to avoid any loss of radioactivity from the stratum corneum. Volunteers were released from the study when radioactivity in the isopropyl alcohol (IPA) swabs of the application area was <5,000 dpm, urine radioactivity was <50 dpm/mL, and fecal radioactivity was <75 dpm/400 mg of homogenized fecal sample. If any of these conditions were not met, the stay of the volunteer was prolonged, and the appropriate test repeated.

C. Sample Collection and Analysis

The dome and bandage were removed after 8 hours of exposure and saved separately for later analysis. The application sites were cleaned with 16 cotton swabs dipped in a 2% solution of Unicura® liquid soap in water. Each swab was saved individually in a glass liquid scintillation vial. The site was then rinsed with a steady stream of soapy water. The volume of the rinse was recorded and 2 aliquots saved in scintillation vials for analysis. After rinsing, the site was dried with two more cotton swabs and then wiped with two cotton swabs soaked in IPA. The application area was then covered with a dry gauze pad until tape stripping. Eighteen hours after cleaning, a 1 x 4-cm section (1/6 of the dose site) was "stripped' with adhesive cellophane tape (3 M Company Scotch® Magic®, 9 mm) and swabbed with IPA to determine the amount of residual radioactivity that was associated with the surface layer of the skin. This process was repeated on another 1 x 4-cm section at 45 hours post application. Sixteen strips were used on

each section. Each stripped was applied evenly to the same area of skin and stripped off in a few seconds. On days 5, 6, and 7 the entire application site was swabbed with 2 cotton swabs soaked in IPA. The site was swabbed daily until the radioactivity was <5,000 dpm. Blood samples were taken from both arms simultaneously, ipsilateral (treated) and contralateral (untreated), at 2, 4, 6, 8, 10, 12, 16, 24, 36, 48, 72, 96, and 120 hours. Total urine volume was collected before dosing and at the intervals 0-4, 4-8, 8-12, 12-24, 24-36, 36-48 hours, and every 24 hours post exposure for 120 hours. Urine was collected after 120 hours only until radioactivity was <50 dpm/mL One pre-dose fecal sample was collected and all post-dose fecal samples were collected for 120 hours. Samples continued to be collected after 120 hours only until radioactivity was <75 dpm. All samples (urine, blood, feces, swabs, skin rinses, tape strips, the dome, template and gauze) were processed accordingly and analyzed for radioactivity using a Beckman liquid scintillation spectrometer.

D. Results

The majority of the dose (60.23%) was found in the skin swab with soapy water and IPA in day 1. The recovery from day 2 to day 7 averaged 0.49%, indicating the removal of the dose after the 8-hour exposure period was effective. The average recoveries of radioactivity as percent of applied dose were 70.54% (swab, skin rinsate, dome, Duoderm®, and gauze pads), 0.89% (tape stripping), 0.0% (feces), and 0.55% (urine). The total average recovery was 71.98%.

The dermal absorption of methamidophos was determined based on the principle used by Feldmann and Maibach (1974) or Wester and Maibach (1985). The method employs the percentage of excreted dose in the urine or feces or both from topical administration and IV dosing. Since there was no recovery of the administered dose in the feces, only the percent of the dose recovered in the urine is used for the estimation of the dermal absorption. Cumulative percentages of the administered dose excreted in the urine are shown in Table 1.

Table 1. Cumulative percentages of dose excreted in the urine after topical administration of methamidophos at 3 $\mu g/cm^2$ in human volunteers.

Post dose interval (hour)	Mean value (%)	Cumulative mean value (%)
0-4	0.01	0.01
4-8	0.03	0.04
8-12	0.03	0.07
12-24	0.08	0.15
24-36	0.09	0.24
36-48	0.06	0.30
48-72	0.11	0.41
72-96	0.07	0.48
96-120	0.06	0.54

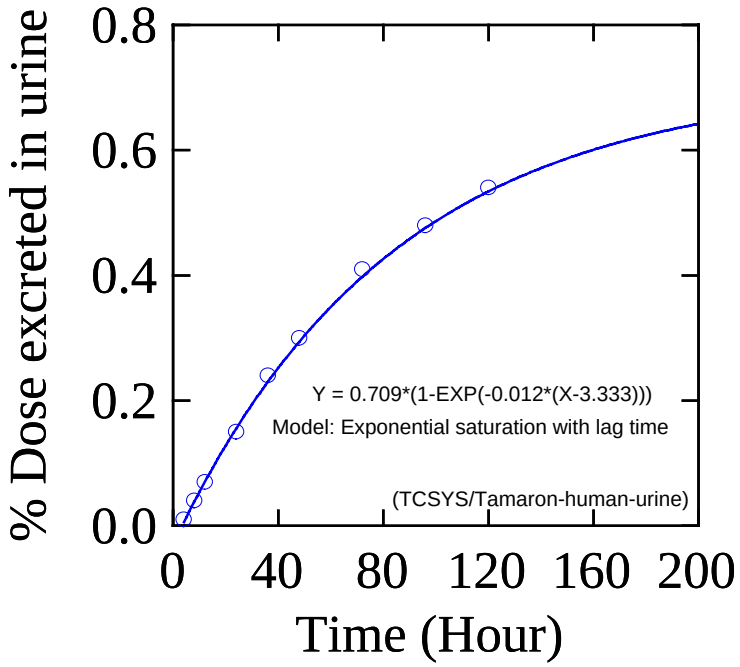
The results indicated that urinary excretion of the administered dose has not reached the plateau at 120 hours post administration. This shows that the excretion of radioactivity was continued after the end of the collection period. The sample collection period should have been longer than 120 hours. Consequently, the maximum excretion of the dose in urine was performed using the exponential saturation model with lag time (Thongsinthusak *et al.*, 1999). The scientific software Systat®, version 8.0 (SPSS, 1998) was utilized for the statistical analysis and plotting a graph. Results in Figure 1 shows the estimated maximum urinary excretion was 0.709%. This percentage of the urinary excretion was used to estimate the dermal absorption.

A study using IV dosing of methamidophos in human volunteers was not conducted. Previously, a study in monkeys using IV dosing was performed (Fuller, 2000). Bayer Corporation noted in the report that it is unacceptable to administer [$^{14}CH_3S$]-methamidophos intravenously to human volunteers. A surrogate study using IV dosing is needed to estimate the dermal absorption of this compound in humans. Wester and Maibach (1993) revealed that dermal absorption values of several chemicals in monkeys and humans are similar. It is assumed that excretion of radioactivity following IV dosing in humans and monkeys would also be similar. Results of the urinary excretion of the dose in monkeys after IV dosing are shown in Table 2.

Table 2. Cumulative urinary excretion of the dose in monkeys following the intravenous bolus dose of methamidophos (46.9 μg/kg body weight).

Post dose interval (hour)	Mean value (%)	Cumulative mean value (%)
0-4	8.22	8.22
4-8	1.84	10.06
8-12	0.28	10.34
12-24	0.38	10.72
24-48	0.22	10.94
48-72	0.19	11.13
72-96	0.12	11.25
96-120	0.10	11.35

Figure 1. Asymptotic plot of cumulative urinary excretion of [$^{14}CH_3S$]-methamidophos after topical administration of 3 µg/cm^2 to human skin.



SYSTAT D:\DATA\TCSYS\Tamaron-Human Urine.SYD,
created Thu Jun 14, 2001 at 15:39:03, contains variables:

TIME RECO

Model: Recov = Max*(1-Exp(-Rate*(Time-Lag)))

Iteration No.	Loss	MAX	RATE	LAG
0	.362331D+00	.500000D+00	.102000D+00	.103000D+00
1	.606771D-01	.455614D+00	.373432D-01	-.370949D+00
2	.192070D-01	.482165D+00	.242531D-01	.729497D+00
3	.634445D-02	.560106D+00	.167438D-01	.392607D+01
4	.100430D-02	.618692D+00	.157792D-01	.424737D+01
5	.517950D-03	.646188D+00	.145907D-01	.403560D+01
6	.316672D-03	.665930D+00	.138192D-01	.382354D+01
7	.228734D-03	.680590D+00	.132787D-01	.365646D+01
8	.190255D-03	.691658D+00	.128915D-01	.352899D+01
9	.174976D-03	.699870D+00	.126162D-01	.343452D+01
10	.170299D-03	.705565D+00	.124313D-01	.336924D+01
11	.169636D-03	.708749D+00	.123303D-01	.333289D+01
12	.169634D-03	.708727D+00	.123319D-01	.333336D+01
13	.169634D-03	.708728D+00	.123319D-01	.333335D+01
14	.169634D-03	.708728D+00	.123319D-01	.333335D+01

Dependent variable is RECOV

Source	Sum-of-Squares	df	Mean-Square
Regression	0.867	3	0.289
Residual	0.000	6	0.000
Total	0.867	9	
Mean corrected	0.309	8	

Raw R-square (1-Residual/Total) = 1.000
Mean corrected R-square (1-Residual/Corrected) = 0.999
R(observed vs predicted) square = 0.999

Parameter	Estimate	A.S.E.	Param/ASE	Wald Confidence Interval Lower < 95%> Upper
MAX	0.709	0.021	33.711	0.657 0.760
RATE	0.012	0.001	18.334	0.011 0.014
LAG	3.333	0.425	7.846	2.294 4.373

The exponential saturation model with lag time (Thongsinthusak *et al.*, 1999) was used to estimate the maximum urinary excretion after the IV administration as it was used to estimate the maximum urinary excretion of the dose after the dermal administration in the volunteers. The estimated maximum urinary excretion of the dose in monkeys after IV dosing was 11.09%. The dermal absorption of methamidophos can be calculated using the equation shown below.

$$\%\ \text{Dermal absorption} = \frac{(\text{Topical})\ ^{14}\text{C in urine (\% dose)}}{(\text{IV})\ ^{14}\text{C in urine (\% dose)}} \times 100$$

$$\%\ \text{Dermal absorption of methamidophos in humans} = \frac{0.709}{11.09} \times 100 = 6.4\%$$

E. Discussion and Conclusion

Bayer Corporation did not formally request scientists at Department of Pesticide Regulation (DPR) to review the dermal absorption study protocol. Upon contact by the representatives of Bayer Corporation about the labeled compound and the low recovery of radioactivity in the urine, the author of this memorandum recommended that ^{32}P-methamidophos should be used (Bayer Corporation, 1998). In 1999, the author was informed that Bayer could not find a lab to synthesize ^{32}P-methamidophos. Later, a lab (person) was found in Russia to synthesize ^{32}P-methamidophos, but that was unreliable. Typically, scientists at DPR recommend a compound that is radiolabeled at the core of the molecule.

Gray *et al*. (1982) conducted a study to determine the distribution and excretion of [$^{14}CH_3S$]-methamidophos in female Sprague-Dawley® rats. The animals were given IV dosing of a non-lethal dose at 8 µg/kg body weight. Within 24 hours of dosing, 47% of the radioactivity was recovered in the urine and 34% as CO_2 with less than 5% in the feces over 7 days. The results indicated that [$^{14}CH_3S$]-methamidophos could be used to study the distribution of this compound in animals because the majority of the dose is excreted in urine. Based on this evidence, it is assumed that the majority of the absorbed dose in humans or monkeys would be excreted in urine and a lower percentage would be converted to CO_2 or volatile components. [$^{14}CH_3S$]-methamidophos was used in dermal absorption studies in monkeys and humans. However, the estimated maximum urinary excretion after IV dosing in monkeys was very low (11.08%) compared to that observed in rats (47%). The average recovery of unabsorbed dose in the dermal absorption study in humans was 70.54%. Approximately, 29% of the administered dermal dose could have been absorbed (indirect estimate). Even though it is more realistic to use a dermal absorption value obtained from a study in humans for exposure assessment, the dermal absorption of 6.4% was estimated based on some assumptions. The dermal absorption of methamidophos could possibly range from 6.4% to 29%. Because of uncertainty for some assumptions, a human dermal absorption value of 6.4% is not recommended for the exposure assessment.

In 1987, a study was conducted to determine the dermal absorption of [$^{14}CH_3S$]-methamidophos in rats. However, this study was unacceptable because the total dose recoveries for several exposure times of the dermal doses were very low (Thongsinthusak, 2001a).

The dermal absorption study of [$^{14}CH_3S$]-methamidophos was conducted in monkeys and completed in 2000. Based on the conditions of this study, the dermal absorption was estimated to be 11.3% (Thongsinthusak, 2001b). This dermal absorption is not recommended for the exposure assessment because the recovery of radioactivity after the IV administration in monkeys was very low, accounting for 11.09%.

The estimated dermal absorption of methamidophos in the human volunteers was 6.4%. This estimate was based on a few assumptions, which may not be accurate. The indirect estimate of the dermal absorption (administered dose – unabsorbed dose) in humans was 29%. This dermal absorption value represents an extreme case scenario. DPR recommends a conservative dermal absorption value of 29% because of uncertainty related to the dermal absorption studies in rats, monkeys and human volunteers.

DPR recommends a new dermal absorption study be conducted by using ^{32}P-methamidophos in animals, such as nonhuman primates. In a dermal absorption study, it is essential that a compound be radiolabeled at a position, which is part of the core of the molecule in order to prevent loss of metabolite(s) due to volatilization. An appropriate dermal dose should be prepared in an aqueous suspension with addition of formulation blank (ingredients used in the methamidophos formulation minus methamidophos). A probe study is recommended. A dermal absorption study protocol should be submitted to DPR for review before the study.

References:

Bayer Corporation. 1998. Dermal absorption of methamidophos: Radiolabeling of methamidophos and recovery of the applied dose. (Personal Communication with representatives of Bayer Corp.).

Feldmann, R. J., and Maibach, H. I. 1974. Percutaneous penetration of some pesticides and herbicides in man. *Toxicol. Appl. Pharmacol*. 28:126-132.

Fuller B. 2000. A dermal/intravenous cross over study to determine the dermal absorption of [^{14}C]-methamidophos in male rhesus monkeys. Bayer Report No. 109812.

Gray, A. J., Thompson, C. M., and Fukuto, T. R. 1982. Distribution and excretion of [$^{14}CH_3S$]-methamidophos after intravenous administration of a toxic dose and the relationship with anticholinesterase activity. *Pestic. Biochem. Physiol*. 18:28-37.

SPSS. 1998. Systat® 8.0. SPSS Inc. Chicago, Illinois.

Thongsinthusak, T., Ross, J. H., Saiz, S. G., and Krieger, R. I. 1999. Estimation of dermal absorption using the exponential saturation model. *Reg. Toxicol. Pharmacol*. 29:37-43.

Thongsinthusak, T. 2001a. Percutaneous absorption of methamidophos in male rats. Worker Health and Safety, Department of Pesticide Regulation. HSM-01006.

Thongsinthusak, T. 2001b. A dermal/intravenous cross over study to determine the dermal absorption of [^{14}C]-methamidophos in male rhesus monkeys. Worker Health and Safety, Department of Pesticide Regulation. HSM-01007

Wester, R. C., and Maibach, H. I. 1985. *In vivo* percutaneous absorption and decontamination of pesticides in humans. *J. Toxicol. Environ. Hlth*. 16:25-37.

Wester, R. C., and Maibach, H. I. 1993. Animal models for percutaneous absorption. In *Health Risk Assessment: Dermal and Inhalation Exposure and Absorption of Toxicants* (R. G. M. Wang, J. B. Knaak, and H. I. Maibach, Eds.), pp. 89-103. CRC Press, Boca Raton, FL.

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